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SIGNALING NETWORKS IN FOCUS

REDOX SIGNALING IN THE CARDIOMYOCYTE: FROM PHYSIOLOGY TO FAILURE

Running title: Redox signaling in the heart

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ABSTRACT

The specific effect of oxygen and reactive oxygen species (ROS) in mediating post-translational modification of protein targets has emerged as a key mechanism regulating signaling components, a process termed redox signaling. ROS act in the post-translational modification of multiple target proteins including receptors, kinases, phosphatases, ion channels and transcription factors. Both O_2 and ROS are major source of electrons in redox reactions in aerobic organisms. Because the heart has the highest O_2 consumption among body organs, it is not surprising that redox signaling is central to heart function and pathophysiology. In this article, we review some of the main cardiac redox signaling pathways and their roles in the cardiomyocyte and in heart failure, with particular focus on the specific molecular targets of ROS in the heart.

SIGNALLING NETWORK FACTS:

- **The view that ROS are either harmful or beneficial is very simplistic. ROS can mediate specific post-translational modifications of biomolecules involved in intracellular signaling networks.**
- **Redox signaling typically involves spatially and temporally confined ROS production by specific sources of ROS, which can amplify or inhibit the activity of signaling network components.**
- **The NADPH oxidase proteins Nox2 and Nox4 are major enzymatic sources of ROS involved in cell signaling in cardiomyocytes.**
- **Redox signaling is involved in acute and chronic cardiac adaptations to diverse stresses and several molecular targets have been identified that are redox-modified in these settings.**

Abbreviations: AKAP, A-kinase anchoring protein; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; ECC, excitation contraction coupling; eIF2α, eukaryotic initiation factor 2α ER, endoplasmic reticulum; ETC, electron transport chain; GPCR, G-protein coupled receptor;

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Hif1, hypoxia-inducible factor 1; HDAC, histone deacetylase; MEF2, myocyte enhancer fator-2; Nox, NADPH oxidase; NO, nitric oxide; NOS, nitric oxide synthase; Nrf2, nuclear factor erythroid-derived 2-like 2; PHD, prolyl hydroxylase; PKA, protein kinase A; PKG, protein kinase G; PLN, phospholamban; PP1, protein phosphatase 1; ROS, reactive oxygen species; RyR, Ryanodine receptor; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; SR, sarcoplasmic reticulum; Trx, thioredoxin

Keywords: Redox signaling; NADPH oxidase; heart failure; reactive oxygen species

1. Introduction

At a time of unprecedented subatomic detail in physics (e.g. the Higgs' boson), it is notable that the chemistry that underpins multi-cellular living systems is the chemistry of the electron. Coupled electron flow involving electron acceptors (e.g. $NAD^+/NADP^+$ and $FAD^+/FADP^+$) and electron donors (e.g. NADH/NADPH and FADH/FADPH) is involved in fundamental cellular processes such as the electron transport chain (ETC) of the mitochondria, protein folding in the endoplasmic reticulum (ER) and many metabolic processes. While there are several primary sources of electrons, the main sources of electrons for redox reactions in aerobic organisms are O_2 itself and O_2-derived reactive species (ROS), because of their abundance and specific chemical proprieties. Perhaps the most abundant O_2-derived ROS is superoxide ($O_2^{\bullet-}$), which may be generated from partial one-electron reduction during electron leak in the mitochondrial ETC as well as by the enzymatic action of several specific oxidases such as NAPDH oxidases (Lassègue et al., 2012), xanthine oxidase (Minhas et al., 2006) and monoamine oxidases (Kaludercic et al., 2010). As a free radical, $O_2^{\bullet-}$ reacts with itself to produce the more stable hydrogen peroxide (H_2O_2) in a reaction that is enhanced many fold by superoxide dismutases. H_2O_2 is a non-radical that can be decomposed by catalase or by oxidizing enzymes such as peroxidases but is also an important oxidizing agent of many organic molecules (e.g. protein cysteine and methionine residues and metalloproteins - see below). O_2 is used by nitric oxide synthases (NOSs) to generate nitric oxide ($^{\bullet}NO$), a reactive nitrogen species (RNS) that is also involved in cell

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signaling. •NO is a precursor of other reactive species, such as $ONOO^-$ (formed by reaction with $O_2^{\bullet-}$) and $ONOOCO_2^-$ (formed in the presence of CO_2/HCO_3) (Augusto et al., 2002).

It has become evident that redox reactions have a key role in cell signaling pathways in addition to their involvement in cellular metabolic and biosynthetic processes. The specific compartmentalized generation of ROS - in particular, H_2O_2 - modulates many cell signaling pathways, a process known as redox signaling. ROS act through post-translational modification of numerous types of biomolecules, particularly proteins such as receptors, kinases, phosphatases, caspases, ion channels and transcription factors. A wide range of redox modifications may be involved in redox signaling, among which the most studied is the H_2O_2-mediated oxidation of cysteine (Cys) residues in proteins. Protein Cys residues with low pKa are susceptible to oxidation to form usually reversible intra- or inter-molecular disulfides (Wouters et al., 2011; Rudyk and Eaton, 2014). ROS-mediated thiol oxidation may modulate the activity of enzymes in which Cys moieties are critical, e.g. by affecting substrate binding in glyceraldehyde 3-phosphate dehydrogenase (Peralta et al., 2015) and many tyrosine phosphatases (Finkel 2011). Cys oxidation may also result in other intermediates, e.g. adducts of Cys with NO (nitrosylation), nitroxyl (HNO; Sivakumaran et al., 2013), or glutathione (glutathiolation) (Rudyk and Eaton, 2014). It is important to note that the effects of ROS are greatly affected by the local anti-oxidant environment. In this regard, glutathione is a major cellular antioxidant that can reduce a wide range of oxidized proteins. The thioredoxin system may have more specific reductive and signaling functions, as discussed in section 3.5.

2. Functions

2.1 Redox signaling in the heart

The beating heart has the highest O_2 consumption of all organs and this can rise several-fold in response to increased workload. Perhaps not surprisingly, along with altered O_2 metabolism, ROS generation and redox signaling are also involved in the cardiac adaptations to different types of physiological and pathological stress (Santos et al., 2011). The heart exhibits acute adaptations in its contractile performance but is also able to chronically remodel its structure and function in the face of prolonged alterations in workload. A virtually universal component of chronic cardiac remodeling is an increase in size of individual cardiomyocytes and the ventricular wall (termed cardiac hypertrophy),

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while sustained disease stress may lead to irreversible structural and contractile dysfunction (i.e. heart failure). Redox signaling pathways play important roles both in acute cardiac adaptations and in chronic cardiac remodeling leading to heart failure (Shah and Mann, 2013). In this article, we summarize current knowledge on the key intracellular molecular targets of redox regulation involved in heart failure. The role of $^{\bullet}$NO and RNS has been reviewed elsewhere (Simon et al., 2014).

2.2 Main sources of ROS in cardiomyocytes

Mitochondria, NADPH oxidases (Noxs), uncoupled NO synthases, xanthine oxidase and monoamine oxidases are among the main ROS sources in the heart (Burgoyne et al., 2012). Mitochondria can generate ROS at different steps during O_2 reduction to H_2O in the ETC. This may be especially important in the setting of ischemia-reperfusion, where ROS generation upon reperfusion was recently shown to be driven by the oxidation of accumulated succinate and consequent reverse electron transport at complex 1 (Chouchani et al., 2014). An additional source of mitochondrial ROS are the monoamine oxidases, which have been implicated in heart failure (Kaludercic et al., 2010; Kaludercic et al., 2014).

Noxs are a family of enzymes that specifically generate ROS as their primary function and have emerged as the major cellular ROS sources involved in redox signaling (Lassègue et al., 2012). Noxs use NADPH as an electron donor to reduce O_2 to $O_2^{\bullet-}$/ H_2O_2 and appear well suited for involvement in redox signaling because (i) they can be specifically activated or expressed by diverse agonists (e.g. G-protein coupled receptor [GPCR] agonists such as angiotensin II) or in specific stress conditions (e.g. hypoxia); (ii) they generate ROS in distinct sub-cellular locations; (iii) they may be co-located with specific signaling targets. Seven Nox isoforms (Nox1-5, Dual oxidase1-2) have been identified, each with a distinct catalytic subunit responsible for oxidase activity and with varying requirements for other accessory subunits. Among these, the Nox2 and Nox4 isoforms are expressed in cardiomyocytes. Both isoforms are transmembrane heterodimers bound to a smaller $p22^{phox}$ subunit but they differ significantly in their sub-cellular location, activation and regulation. Nox2 is located at the sarcolemma and in t-tubules. It requires acute activation by agonists (e.g. angiotensin II, mechanical forces) in a complex process that involves the post-translation modification of regulatory subunits ($p47^{phox}$, $p67^{phox}$, $p40^{phox}$, Rac1) and their association with the Nox2-$p22^{phox}$ heterodimer to form the fully activated enzyme. By contrast, Nox4 is found in the

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endoplasmic reticulum (sarcoplasmic reticulum [SR] in cardiomyocytes) and possibly the mitochondria. Intriguingly, a splice variant of Nox4, termed Nox4D, is located in the nucleus (Anilkumar et al., 2013). Nox4 is constitutively active (i.e., does not require acute activation) and is thought to be regulated predominantly by its abundance and location. Nox2 and Nox4 have distinct and often contrasting effects in the pathophysiology of heart failure (Burgoyne et al., 2012). Detailed reviews of the regulation of Noxs and their roles in the cardiovascular system have been published recently (Lassègue et al., 2012; Brandes et al., 2014).

3. Cascades and Key Molecules

Several different molecular targets of redox regulation in heart failure have been identified. Here we consider the key targets for which molecular mechanisms of redox modulation have been identified (Fig. 1).

3.1 Ca^{2+}/calmodulin-dependent kinase II (CaMKII).

CaMKII is a multifunctional kinase present as γ, β or δ isoforms. The transient activation of CaMKII-δ in cardiomyocytes has been implicated in physiological β-adrenergic agonist-induced increases in heart rate and contractility. However, sustained CaMKII activation may be detrimental via effects on excitation-contraction coupling (ECC) and cell death, and may contribute to heart failure (Luczak et al., 2014). CaMKII is a supramolecular complex formed of 12 monomers assembled as hexamers, with each monomer having catalytic, auto-inhibitory and Ca/calmodulin-binding domains. Under resting conditions the auto-inhibitory domain mimics substrate binding to the catalytic domain and blocks kinase activity. The binding of Ca^{2+} to the Ca/calmodulin domain induces auto-phosphorylation and disrupts the auto-inhibitory domain to expose the catalytic site and maintain the enzyme functional even when Ca^{2+} levels decline. The Anderson laboratory found that following initial Ca-dependent activation of CamKII, the specific oxidation of conserved Met 281/282 residues in the regulatory domain could increase CaMKII activity independent of Ca/calmodulin (Erickson et al., 2008). Furthermore, the Met oxidation could be reversed by methionine sulfoxide reductase A, supporting a physiological role for this modification. CaMKII can through this mechanism therefore integrate two key signaling molecules, namely Ca^{2+} and ROS. Studies in gene-modified mice showed that CaMKII oxidation contributes to an increase in cardiomyocyte death and the development of heart failure after myocardial infarction or

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chronic angiotensin II stresss (Erickson et al., 2008). Subsequent studies have confirmed a role of increased CaMKII oxidation in several cardiac stress situations (Wagner et al., 2006, Li et al., 2012; Purohit et al., 2013).

Nox2 was found to be the upstream mediator of CaMKII oxidation during chronic neurohumoral stress, with mice deficient in Nox2 oxidase activity showing reduced cardiomyocyte apoptosis and heart failure in models of chronic angiotensin II infusion and myocardial infarction (Erickson et al., 2008). Nox2 was also linked to CaMKII activation in a model of endoplasmic reticulum stress-induced cardiac dysfunction (Roe and Ren, 2013) and in a cellular model of angiotensin II-induced arrhythmia (Wagner et al., 2014). On the other hand, in a mouse model of streptozotocin-induced diabetes, cardiac bradycardia following myocardial infarction was related to increased mitochondrial-derived ROS and hyperactivity of oxidised CaMKII (Luo et al., 2013). Therefore, it appears that different ROS sources can link to CaMKII oxidation depending on the context. More recently, it has been reported that an increase in CaMKII activity can be induced via O-GlcNAc-modification at Ser 279, which similar to the oxidized phenotype exhibits molecular memory in that enzyme activity is maintained even after a decline in Ca^{2+} concentration (Erickson et al., 2013). The latter modification was linked to cardiac arrhythmia in the diabetic heart.

3.2 cGMP-dependent protein kinase (PKG).

PKG is a cytosolic protein that forms a homodimer of two identical subunits folded together by a leucine zipper interaction at the N-terminal, a site that determines PKG interaction to substrates (G-Kinase anchoring proteins). The binding of cGMP to a regulatory domain exposes the catalytic domain and kinase activity. In cardiac cells, PKG type 1 has at least 5 Cys residues that can be oxidized. The oxidation of Cys42 results in an inter-disulfide between the two monomers which activates the kinase independently of the NO-cGMP pathway (Burgoyne et al., 2007). The Eaton laboratory developed a "redox-dead" PKG1α^{C42S} knock-in mouse model and found that these animals developed modest hypertension, suggesting that PKG oxidation and resulting vasodilation may be involved in the physiological regulation of blood pressure (Prysyazhna et al., 2012). Increased PKG activity has generally been reported to be cardioprotective in response to hemodynamic stresses and, therefore, in contrast to oxidation-induced increases in CaMKII and PKA activity (see later), it might be anticipated that PKG oxidation might be beneficial during heart failure.

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However, it was recently found that “redox-dead" PKG1α^{C42S} knock-in mice (in which PKG oxidation is inhibited) were actually relatively protected against the development of hemodynamic stress-induced heart failure (Nakamura et al., 2015). It was suggested that PKG1 oxidation during hemodynamic stress leads to failure of translocation of the enzyme to the sarcolemma where it normally mediates anti-hypertrophic signaling by inhibiting transient receptor potential channel 6 (TRPC6) (Nakamura et al., 2015). The ROS sources responsible for PKG oxidation are yet to be determined. These results suggest that the modification of substrate binding (and therefore subcellular location) subsequent to change in PKG1 redox state is as important as changes in activity *per se*. The maintenance of PKG in a reduced state during chronic hemodynamic stress therefore appears to be beneficial.

3.3 cAMP-dependent protein kinase (PKA).

β-adrenergic stimulation stimulates adenylate cyclase to increase intracellular cAMP levels and activate PKA, which in turn phosphorylates several important proteins involved in ECC (e.g. the L-type Ca channel, phospholamban and troponin I). As such, PKA is a central regulator of cardiac contractility. Furthermore, altered PKA activity is involved in the pathophysiology of heart failure. PKA is a hetero-tetramer with a regulatory subunit dimer and a catalytic subunit dimer. It was found that PKA oxidation resulting in the formation of an inter-disulfide bond between the two regulatory subunits activates the enzyme independently of cAMP (Brennan et al., 2006). Furthermore, this modification increases the affinity for A-kinase anchoring proteins (AKAPs) which targets the kinase to its substrates, thereby causing PKA translocation. Recently, it was shown with the use of a novel mouse model in which PKA is "redox-dead" due to a Cys175Ser mutation in the R subunit, that this disrupted PKA-dependent VEGF/ERK signaling (Burgoyne et al., 2015). The functional consequence of PKA oxidation was found to be an enhancement of angiogenesis in models of hind-limb ischemia and tumor angiogenesis, and this work suggested that Nox4 may be upstream of PKA oxidation. It will be of interest in future studies to establish what role(s) PKA oxidation may play in heart physiological function and failure, and what ROS sources are involved.

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3.4 Proteins involved in Excitation Contraction Coupling (ECC).

During cardiomyocyte ECC, initial influx of Ca^{2+} through sarcolemmal L-type Ca^{2+} channels following membrane depolarization induces the opening of ryanodine receptor (RyR) channels in the SR and Ca^{2+} release into cytoplasm (Ca-induced Ca-release) to promote contraction upon the binding of Ca^{2+} to troponin C. Muscle relaxation is promoted by the re-uptake of Ca^{2+} into the SR via the SR Ca^{2+}-ATPase pump (SERCA) and Ca^{2+} efflux from the cell via the Na^{+}/Ca^{2+} exchanger. The affinity of SERCA for Ca^{2+} is regulated by its interaction with phospholamban (PLN) (Kranias and Hajjar, 2012). In addition to the fundamental physiological role of ECC in mediating acute changes in heart contractile performance, abnormalities of ECC are a key pathogenic feature of heart failure (Kohler et. al., 2014). Many key proteins involved in ECC, e.g. the L-type Ca channel, RyR, SERCA, PLN and troponin I, are regulated by phosphorylation by PKA, CaMKII or PKG and may therefore be indirect targets of redox signaling (Fig. 2). Recently, we found an indirect redox modulation of SERCA activity mediated via Nox2 (Zhang et al., 2015). In hearts overexpressing Nox2, SERCA activity and contractile performance were increased as a result of an increase in PLN phosphorylation. This increase in PLN phosphorylation was associated with a decrease in activity of the Ser/Thr protein phosphatase-1 (PP1), which normally dephosphorylates PLN. This finding highlights the fact that redox regulation of phosphorylation may occur secondary to reduced phosphatase activity as well as changes in kinase activity.

Some proteins involved in ECC, such as RyR and SERCA, are also direct targets of ROS. The RyR structure was recently resolved by electron microscopy and comprises a tetrameric complex (Zalk et al., 2015). Each subunit has around 20 free Cys residues that can be subject to various redox modifications. It was recently shown that mechanical stretch of cardiomyocytes induces cyclical activation of Nox2 in t-tubules and leads to an augmentation of RyR Ca^{2+} release (Ca^{2+} sparks), thereby contributing to a fundamentally important physiological pathway for stretch-induced augmentation of cardiac output (Prosser et al., 2011; Prosser et al., 2013). At a molecular level, this is likely to be mediated by reversible oxidation of the RyR although this was not definitively demonstrated in this study. In pathological settings, more extensive/sustained and often irreversible oxidation or hyper-nitrosylation of the RyR contributes to Ca^{2+} leak from the SR, with consequent increase in arrhythmia and contractile dysfunction (Simon et al., 2014). The precise ROS sources that may be involved in RyR oxidation in heart failure remain unclear. Mitochondria

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are likely to be an important source while Nox2 is also implicated in models of muscular dystrophy (Prosser et al., 2011). In skeletal muscle, it was reported that Nox4 can oxidize RyR1 and lead to increased Ca^2 leak during hypoxia (Sun et al., 2011). Whether Nox4 has similar effects in cardiac myocytes is not known.

SERCA activity is also redox modulated. In vascular smooth muscle, the reversible glutathionylation of Cys674 increases SERCA activity and induces vasorelaxation independent of NO-cGMP (Adachi et al., 2004). Physiological redox regulation of SERCA in the heart remains to be definitively established. However, SERCA2 activity may be enhanced by HNO-induced oligomerization of PLN, which reduces the amount of inhibitory monomeric PLN (Sivakumaran et al., 2013). Irreversible oxidation of cardiac muscle SERCA in disease settings such as hemodynamic overload and chronic neurohumoral activation impairs activity and causes contractile dysfunction (Lancel et al., 2010; Qin et al., 2014).

3.5 Molecules involved in cardiac hypertrophy and remodeling.

The redox-sensitive modulation of the small G protein Ras, kinases such as ERK1/2, p38MAPK, protein kinase C and Akt, and transcription factors such as AP-1 and NF-κB, is well established in many different body systems and is not considered in detail here. However, it has been found that Nox2-dependent activation of ERK1/2 and Akt contribute to the development of GPCR agonist-induced cardiac hypertrophy. (Bendall et al., 2002; Satoh et al., 2006; Burgoyne et al., 2012) The redox activation of apoptosis signaling kinase-1 and p38MAPK/JNK (most likely via Nox2) contributes to cardiomyocyte cell death and detrimental ventricular remodeling in heart failure (Yamaguchi et al., 2003).

Thioredoxin (Trx) is an important molecule that mediates the redox shuffling of protein thiols in different cell compartments (Trx1 in the cytoplasm and Trx2 in the mitochondria). Trx has a conserved motif of two vicinal Cys which can form an intramolecular disulfide bond. Trx reduces oxidized thiols in target proteins, typically via specific protein-protein interaction, and itself becomes oxidized in the process. The oxidized Trx is reduced back by thioredioxin reductase in an NADPH and FAD-dependent reaction. The Trxs and peroxiredoxins (which react avidly with H_2O_2) may function as redox relays for the transmission of H_2O_2 signals, e.g. in the regulation of transcription factors (Jarvis et al., 2012; Sobotta et al., 2015). Dysfunctional Trx1 and Trx2 are implicated in increased cardiac

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ROS production and cardiac remodeling, while overexpression of Trx1 attenuated pressure overload-induced cardiac hypertrophy (Yamamoto et al., 2003; Stanley et al., 2011).

Another molecular target of redox regulation during cardiac hypertrophy is the Class II histone deacetylase, HDAC4, which is known to be important in inhibiting the expression of pro-hypertrophic genes induced by the transcription factor myocyte enhancer fator-2 (MEF2). HDAC4 phosphorylation promotes its export from the nucleus, thereby facilitating hypertrophic gene expression. Ago et al. (Ago et al., 2008) showed that phosphorylation-independent nuclear export of HDAC4 and induction of hypertrophy resulted from specific Trx1-sensitive HDAC4 oxidation (intramolecular disulfide formation between Cys-667 and Cys-669) during α-adrenoceptor stimulation (Fig. 1). Nox4 was reported to be a ROS source that mediates HDAC4 oxidation and cardiac hypertrophy (Matsushima et al., 2013). Isoproterenol-induced export of HDAC5 from the cardiomyocyte nucleus was also reported to be mediated by oxidation (Haworth et al., 2012). Class II HDACs are phosphorylated by CaMKII or PKA during heart failure (Backs et al., 2001) and therefore can also be an indirect target of redox regulation (Fig. 1). Taken together, these data highlight the potential for redox regulation to add a layer of complexity that fine tunes the normal epigenetic regulation modulated by phosphorylation and acetylation.

3.6 Molecules involved in other cardiac stress responses.

Prolyl hydroxylases (PHDs) are enzymes that use O_2 to hydroxylate proline residues in proteins. Proline hydroxylation of the transcription factor Hif1 (hypoxia-inducible factor 1) is a major mechanism that regulates Hif stability by promoting its proteosomal degradation. In the heart, Hif1α is induced by hypoxia and chronic hemodynamic overload and drives diverse gene programs, e.g. the promotion of angiogenesis. It has been shown that Hif1α-VEGF signaling is important in driving coordinated heart hypertrophy and angiogenesis that promotes preservation of cardiac function during chronic pressure overload (Shiojima et al., 2005; Sano et al., 2007). Hypoxia as well as ROS can inhibit PHDs and thereby stimulate Hif1α-VEGF signaling (Lee and Simon, 2015). Nox4 expression is upregulated by hypoxia and chronic hemodynamic overload and studies in gene-modified mouse models showed that Nox4 is protective against cardiac decompensation during hemodynamic overload (Zhang et al., 2010). We found that this protection was related to an enhanced Hif1α activation and

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release of VEGF from cardiomyocytes (most likely due to PHD inhibition), resulting in paracrine angiogenic activity that facilitated the preservation of myocardial capillary density during pressure overload (Zhang et al., 2010). A similar Nox4-dependent activation of Hif1α has been demonstrated in the setting of cardiac ischemia (Matsushima et al., 2013) but mitochondrial ROS can also activate Hif1 (Chandel et al., 1998). It is interesting to note, however, that long-term inhibition of PHD activity may lead to cardiomyopathy (Moslehi et al., 2010). This may relate to the adverse effects of prolonged Hif1α activation or to other targets regulated by PHDs. In this context, it was recently discovered that thyroid hormone receptor-α, a transcriptional regulator of many genes, is hydroxylated by PHD2/3 in the heart (Xie et al., 2015). The inhibition of PHD led to an increase in thyroid hormone receptor-α-dependent suppression of PLN gene expression, a sustained activation of CaMKII and a sensitization of mice to chronic β-adrenergic stress-induced myocardial injury (Xie et al., 2015).

Other targets of Nox-mediated redox signaling in the setting of acute and chronic cardiac stress have also been identified. The transcription factor Nrf2 (nuclear factor erythroid-derived 2-like 2) is a master regulator of a cytoprotective gene program that is activated in cardiac myocytes during acute neurohumoral stress or in the heart *in vivo* during chronic hemodynamic overload, settings in which Nox4 levels also increase. The upregulation of Nrf2 in these settings was found to be under the specific regulation of Nox4 and Nrf2 contributed to the cardioprotective effects of Nox4 (Brewer et al. 2011; Smyrnias et al., 2015). Nox4 is also upregulated during protein unfolding stress in cardiac cells and we have recently identified that it boosts stress-induced phosphorylation of the eukaryotic initiation factor 2α (eIF2α) and the so-called integrated stress response via a very specific and localized redox inhibition of PP1 at the ER (Santos et al., 2016). PP1 inhibition by Nox4 involves the oxidation of its metal center rather than cysteine oxidation (Santos et al., 2016). This Nox4-mediated enhancement of eIF2α signaling mediates cardioprotective effects by reducing myocardial infarct size during acute ischemia-reperfusion (Santos et al., 2016). Ischemic cardiac preconditioning is a phenomenon whereby transient exposure to ischemia induces protection against subsequent prolonged ischemia. Here, it has been found that Nox2-derived ROS can trigger ischemic cardiac preconditioning through protein kinase C activation (Bell et al., 2005; Kimura et al., 2005).

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4. Associated pathologies and therapeutic implications

While oxidative stress has been implicated in the pathophysiology of heart disease for several decades, only more recently have the role of redox signaling and specific molecular targets of such signaling become better appreciated. Among the specific molecular targets that can become redox-modified through specific post-translational modifications, a number (e.g. CaMKII and other kinases) may act as hubs in signaling networks through their ability to integrate different types of signals, e.g. Ca^{2+}, cyclic nucleotide second messengers and ROS generated by specific enzymes. Specific ROS sources such as Nox2 and Nox4 may themselves integrate the effects of different stresses as a consequence of stress-induced activation or increase in abundance. Such redox hubs may be especially important in the context of heart failure. A striking observation is that redox circuits can mediate adaptive or detrimental effects, indicating one reason why non-specific "antioxidant" approaches have failed in heart disease and why it may be better to identify specific targets for therapeutic manipulation. Targets for inhibition may include Nox2 and CamKII, both of which appear to contribute to the development of heart failure, whereas the activation of other targets (e.g. Nox4-regulated pathways) might be beneficial. Several important challenges remain. We need to elucidate how different redox molecular mechanisms interact with other post-translational modifications, cell compartment-specific effects, the interplay among different ROS sources, and the integrated functioning of different signaling pathways under normal and stress condition. Better tools to monitor ROS production and action (e.g. probes for real-time detection of ROS at high spatial and temporal resolution) as well as systems biology approaches to elucidate integrated signaling will be valuable. Finally, more human data is required to provide clinical relevance and determine the potential for clinical translation.

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References

Adachi T, Weisbrod RM, Pimentel DR, Ying J, Sharov VS, Schöneich C, et al. S-Glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide. Nature Medicine 2004;10: 1200-1207.

Ago T, Liu T, Zhai P, Chen W, Li H, Molkentin JD, et al. A redox-dependent pathway for regulating class II HDACs and cardiac hypertrophy. Cell 2008;133:978-993.

Anilkumar N, San-Jose G, Sawyer I, Santos CX, Sand C, Brewer AC, et al. A 28-kDa splice variant of NADPH oxidase-4 is nuclear-localized and involved in redox signaling in vascular cells. Arteriosclerosis Thrombosis and Vascular Biology 2013;33:104-112.

Augusto O, Bonini MG, Amanso AM, Linares E, Santos CC, De Menezes SL. Nitrogen dioxide and carbonate radical anion: two emerging radicals in biology. Free Radical Biology and Medicine 2002;32:841-859.

Backs J, Worst BC, Lehmann LH, Patrick DM, Jebessa Z, Kreusser MM, et al. Selective repression of MEF2 activity by PKA-dependent proteolysis of HDAC4. Journal of Cell Biology 2011;195:403-415.

Bell RM, Cave AC, Johar S, Hearse DJ, Shah AM, Shattock MJ. Pivotal role of NOX-2 containing NADPH oxidase in early ischemic preconditioning. FASEB Journal 2005;19:2037-2039.

Bendall JK, Cave AC, Heymes C, Gall N, Shah AM. Pivotal role of a gp91(phox)-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. Circulation 2002;105:293-296.

Brandes RP, Weissmann N, Schröder K. Redox-mediated signal transduction by cardiovascular Nox NADPH oxidases. Journal of Molecular and Cellular Cardiology 2014;73:70-79.

ACCEPTED MANUSCRIPT

Brennan JP, Bardswell SC, Burgoyne JR, Fuller W, Schröder E, Wait R, et al. Oxidant-induced activation of type I protein kinase A is mediated by RI subunit interprotein disulfide bond formation. Journal of Biological Chemistry 2006;281:21827-21836.

Brewer AC, Murray TV, Arno M, Zhang M, Anilkumar NP, Mann GE, Shah AM. Nox4 regulates Nrf2 and glutathione redox in cardiomyocytes in vivo. Free Radical Biology and Medicine 2011;51:205-215.

Burgoyne JR, Madhani M, Cuello F, Charles RL, Brennan JP, Schröder E, et al. Cysteine redox sensor in PKGIa enables oxidant-induced activation. Science 2007;317:1393-1397.

Burgoyne JR, Mongue-Din H, Eaton P, Shah AM. Redox signaling in cardiac physiology and pathology. Circulation Research 2012;111:1091-1106.

Burgoyne JR, Rudyk O, Cho HJ, Prysyazhna O, Hathaway N, Weeks A, et al. Deficient angiogenesis in redox-dead Cys17Ser PKARIα knock-in mice. Nature Communications 2015;10:7920-7928.

Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proceedings of the National Academy of Sciences of the USA 1998;95:11715-11720.

Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 2014;515:431-435.

Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, et al. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell 2008;133:462-474.

Erickson JR, Pereira L, Wang L, Han G, Ferguson A, Dao K, et al. Diabetic hyperglycaemia activates CaMKII and arrhythmias by O-linked glycosylation. Nature 2013;502:372-376.

ACCEPTED MANUSCRIPT

Finkel T. Signal transduction by reactive oxygen species. Journal of Cell Biology 2011;194:7-15.

Haworth RS, Stathopoulou K, Candasamy AJ, Avkiran M. Neurohormonal regulation of cardiac histone deacetylase 5 nuclear localization by phosphorylation-dependent and phosphorylation-independent mechanisms. Circulation Research 2012;110:1585-1595.

Jarvis RM, Hughes SM, Ledgerwood EC. Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. Free Radical Biology and Medicine 2012;53:1522-1530.

Kaludercic N, Takimoto E, Nagayama T, Feng N, Lai EW, Bedja D, et al. Monoamine oxidase A-mediated enhanced catabolism of norepinephrine contributes to adverse remodeling and pump failure in hearts with pressure overload. Circulation Research 2010;106:193-202.

Kaludercic N, Carpi A, Nagayama T, Sivakumaran V, Zhu G, Lai EW, et al. Monoamine oxidase B prompts mitochondrial and cardiac dysfunction in pressure overloaded hearts. Antioxidants and Redox Signaling 2014;20:267-80.

Kimura S, Zhang GX, Nishiyama A, Shokoji T, Yao L, Fan YY, et al. Role of NAD(P)H oxidase- and mitochondria-derived reactive oxygen species in cardioprotection of ischemic reperfusion injury by angiotensin II. Hypertension 2005;45:860-866.

Köhler AC, Sag CM, Maier LS. Reactive oxygen species and excitation-contraction coupling in the context of cardiac pathology. Journal of Molecular and Cellular Cardiology 2014;73:92-102.

Kranias EG, Hajjar RJ. Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome. Circulation Research 2012;110:1646-1660.

ACCEPTED MANUSCRIPT

Lancel S, Qin F, Lennon SL, Zhang J, Tong X, Mazzini MJ, et al. Oxidative posttranslational modifications mediate decreased SERCA activity and myocyte dysfunction in Galphaq-overexpressing mice. Circulation Research 2010;107:228-232.

Lassègue B, San Martín A, Griendling KK. Biochemistry, physiology, and pathophysiology of NADPH oxidases in the cardiovascular system. Circulation Research 2012;110:1364-1390.

Lee KE, Simon MC. Snapshot: Hypoxia-Inducible Factors. Cell 2015;163:1288.

Li N, Wang T, Wang W, Cutler MJ, Wang Q, Voigt N, et al. Inhibition of CaMKII phosphorylation of ryr2 prevents induction of atrial fibrillation in fkbp12.6 knockout mice. Circulation Research 2012;110:465-470.

Luczak ED, Anderson ME. CaMKII oxidative activation and the pathogenesis of cardiac disease. Journal of Molecular and Cellular Cardiology 2014;73:112-116.

Luo M, Guan X, Luczak ED, Lang D, Kutschke W, Gao Z, et al. Diabetes increases mortality after myocardial infarction by oxidizing CaMKII. Journal of Clinical Investigation 2013;123:1262-1274.

Matsushima S, Kuroda J, Ago T, Zhai P, Park JY, Xie LH, et al. Increased oxidative stress in the nucleus caused by Nox4 mediates oxidation of HDAC4 and cardiac hypertrophy. Circulation Research 2013;112:651-663.

Matsushima S, Kuroda J, Ago T, Zhai P, Ikeda Y, Oka S, et al. Broad suppression of NADPH oxidase activity exacerbates ischemia/reperfusion injury through inadvertent downregulation of hypoxia-inducible factor-1α and upregulation of peroxisome proliferator-activated receptor-α. Circulation Research 2013;112:1135-1149.

Minhas KM, Saraiva RM, Schuleri KH, Lehrke S, Zheng M, Saliaris AP, et al. Xanthine oxidoreductase inhibition causes reverse remodeling in rats with dilated cardiomyopathy. Circulation Research 2006;98:271-279.

ACCEPTED MANUSCRIPT

Moslehi J, Minamishima YA, Shi J, Neuberg D, Charytan DM, Padera RF, et al. Loss of hypoxia-inducible factor prolyl hydroxylase activity in cardiomyocytes phenocopies ischemic cardiomyopathy. Circulation 2010;122:1004–1016.

Nakamura T, Ranek MJ, Lee DI, Shalkey-Hahn V, Kim C, Eaton P, et al. Prevention of PKG1α oxidation augments cardioprotection in the stressed heart. Journal of Clinical Investigation 2015;125:2468 24672.

Peralta D, Bronowska AK, Morgan B, Dóka É, Van Laer K, Nagy P, et al. A proton relay enhances H_2O_2 sensitivity of GAPDH to facilitate metabolic adaptation. Nature Chemical Biology 2015;11:156-163.

Prosser BL, Ward CW, Lederer WJ. X-ROS signaling: Rapid mechano-chemo transduction in heart. Science 2011;333:1440-1445

Prosser BL, Khairallah RJ, Ziman AP, Ward CW, Lederer WJ. X-ROS signaling in the heart and skeletal muscle: stretch-dependent local ROS regulates $[Ca^{2+}]i$. Journal of Molecular and Cellular Cardiology 2013;58:172-181.

Prysyazhna O, Rudyk O, Eaton P. Single atom substitution in mouse protein kinase G eliminates oxidant sensing to cause hypertension. Nature Medicine 2012;18:286-290.

Purohit A, Rokita AG, Guan X, Chen B, Koval OM, Voigt N, et al. Oxidized CaMKII triggers atrial fibrillation. Circulation 2013;128:1748-1757.

Qin F, Siwik D, Pimentel DR, Morgan R, Biolo A, Tu VH, et al. Cytosolic H_2O_2 mediates hypertrophy, apoptosis, and decreased SERCA activity in mice with chronic hemodynamic overload. American Journal of Physiology - Heart and Circulatory Physiology 2014;306:H1453-H1463.

ACCEPTED MANUSCRIPT

Roe ND, Ren J. Oxidative activation of Ca2+/calmodulin-activated kinase II mediates ER stress-induced cardiac dysfunction and apoptosis. American Journal of Physiology - Heart and Circulatory Physiology 2013;304:H828-H839.

Rudyk O, Eaton P. Biochemical methods for monitoring protein thiol redox states in biological systems. Redox Biology 2014;2:803-813.

Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, et al. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. Nature 2007;446:444-448.

Santos CX, Anilkumar N, Zhang M, Brewer AC, Shah AM. Redox signaling in cardiac myocytes. Free Radical Biology and Medicine 2011;50:777-793.

Santos CX, Hafstad AD, Berreta M, Zhang M, Molenaar C, Kopec J, et al. Targeted redox inhibition of protein phosphatase-1 by Nox4 regulates eIF2α-mediated stress siganling. European Molecular Biology Organisation Journal 2016; DOI 10.15252/embj.201592394 Published online 07.01.2016.

Satoh M, Ogita H, Takeshita K, Mukai Y, Kwiatkowski DJ, Liao JK. Requirement of Rac1 in the development of cardiac hypertrophy. Proceedings of the National Academy of Sciences of the USA 2006;103:7432-7437.

Shah AM, Mann DL. In search of new therapeutic targets and strategies for heart failure: recent advances in basic science. Lancet 2011;378:704-712.

Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, et al. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. Journal of Clinical Investigation 2005;115:2108-2118.

ACCEPTED MANUSCRIPT

Simon JN, Duglan D, Casadei B, Carnicer R. Nitric oxide synthase regulation of cardiac excitation-contraction coupling in health and disease. Journal of Molecular and Cellular Cardiology 2014;73:80-91

Sivakumaran V, Stanley BA, Tocchetti CG, Ballin JD, Caceres V, Zhou L, et al. HNO enhances SERCA2a activity and cardiomyocyte function by promoting redox-dependent phospholamban oligomerization. Antioxidant and Redox Signaling 2013;19:1185-1197.

Smyrnias I, Zhang X, Zhang M, Murray TV, Brandes RP, Schröder K, et al. Nicotinamide adenine dinucleotide phosphate oxidase-4-dependent upregulation of nuclear factor erythroid-derived 2-like 2 protects the heart during chronic pressure overload. Hypertension 2015;65:547-553.

Sobotta MC, Liou W, Stöcker S, Talwar D, Oehler M, Ruppert T, et al. Peroxiredoxin-2 and STAT3 form a redox relay for H_2O_2 signaling. Nature Chemical Biology 2015;11:64-70.

Stanley BA, Sivakumaran V, Shi S, McDonald I, Lloyd D, Watson WH, et al. Thioredoxin reductase-2 is essential for keeping low levels of H_2O_2 emission from isolated heart mitochondria. Journal of Biological Chemistry 2011;286:33669-33677.

Sun QA, Hess DT, Nogueira L, Yong S, Bowles DE, Eu J, et al. 2011. Oxygen-coupled redox regulation of the skeletal muscle ryanodine receptor-Ca2+ release channel by NADPH oxidase 4. Proceedings of the National Academy of Sciences of the USA 2011;108:16098-16103.

Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, et al. 2006. Ca2+/calmodulin-dependent protein kinase II regulates cardiac Na+ channels. Journal of Clinical Investigation 2006;116: 3127-3138.

Wagner S, Dantz C, Flebbe H, Azizian A, Sag CM, Engels S, et al. NADPH oxidase 2 mediates angiotensin II-dependent cellular arrhythmias via PKA and CaMKII. Journal of Molecular and Cellular Cardiology 2014;75:206-15.

ACCEPTED MANUSCRIPT

Wouters MA, Iismaa S, Fan SW, Haworth NL. Thiol-based redox signalling: rust never sleeps. International Journal of Biochemistry and Cell Biology 2011;43:1079-1085.

Xie L, Pi X, Townley-Tilson WH, Li N, Wehrens XH, Entman ML, et al. PHD2/3-dependent hydroxylation tunes cardiac response to β-adrenergic stress via phospholamban. Journal of Clinical Investigation 2015;125:2759-2771.

Yamaguchi O, Higuchi Y, Hirotani S, Kashiwase K, Nakayama H, Hikoso S, et al. 2003. Targeted deletion of apoptosis signal-regulating kinase 1 attenuates left ventricular remodeling. Proceedings of the National Academy of Sciences of the USA 2003;100:15883-15888.

Yamamoto M, Yang G, Hong C, Liu J, Holle E, Yu X, et al. Inhibition of endogenous thioredoxin in the heart increases oxidative stress and cardiac hypertrophy. Journal of Clinical Investigation 2003;112:1395-1406.

Zalk R, Clarke OB, des Georges A, Grassucci RA, Reiken S, Mancia F, et al. Structure of a mammalian ryanodine receptor. Nature 2015;517:444-449.

Zhang M, Brewer AC, Schröder K, Santos CX, Grieve DJ, Wang M, et al. NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. Proceedings of the National Academy of Sciences of the USA 2010;107:18121-18126.

Zhang M, Prosser BL, Bamboye MA, Gondim AN, Santos CX, Martin D, et al. Contractile Function During Angiotensin-II Activation: Increased Nox2 Activity Modulates Cardiac Calcium Handling via Phospholamban Phosphorylation. Journal of the American College of Cardiology 2015;66:261-272.

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FIGURE LEGENDS

Fig. 1. Schematic showing stress-induced signalling cascades in the heart. The major stress stimuli that induce chronic adaptation of the heart are neurohumoral agonists, cytokines, growth factors, mechanical forces and hypoxia. Key kinases and enzymes that are redox-sensitive include MAPKs, protein kinase C (PKC), protein kinase G (PKG), protein kinase A (PKA), Ca/calmodulin kinase II (CamKII) and HIF prolyl hydroxylases (PHD). Class II histone deacetylases (HDAC) can also be redox-modulated. These eventually affect the transcription of genes that contribute to hypertrophy, metabolic changes, cytoprotection, angiogenesis and other changes.

Fig. 2. Acute redox-sensitive stress responses that affect cardiomyocyte excitation-contraction coupling (ECC) and calcium homeostasis. Cardiomyocyte ECC involves Ca-induced Ca release from the sarcoplasmic reticulum (SR) via Ryanodine receptors (RyR), to activate myofilaments. Ca is taken back into the SR via the SERCA pump, which is regulated by phospholamban (PLB). Other channels and pumps contributing to ECC include Na and K channels, the Na/Ca exchanger (NCX) and the sarcolemmal Ca ATPase. Beta-adrenergic stimulation is a major modulator of ECC through the cAMP/PKA-dependent phosphorylation of PLB, the L-type Ca channel and myofilaments. Redox modulation of PKA, PKG and CamKII as well as direct effects of ROS on the RyR synergise with the Ca-dependent and beta-adrenergic regulation.

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Fig. 1

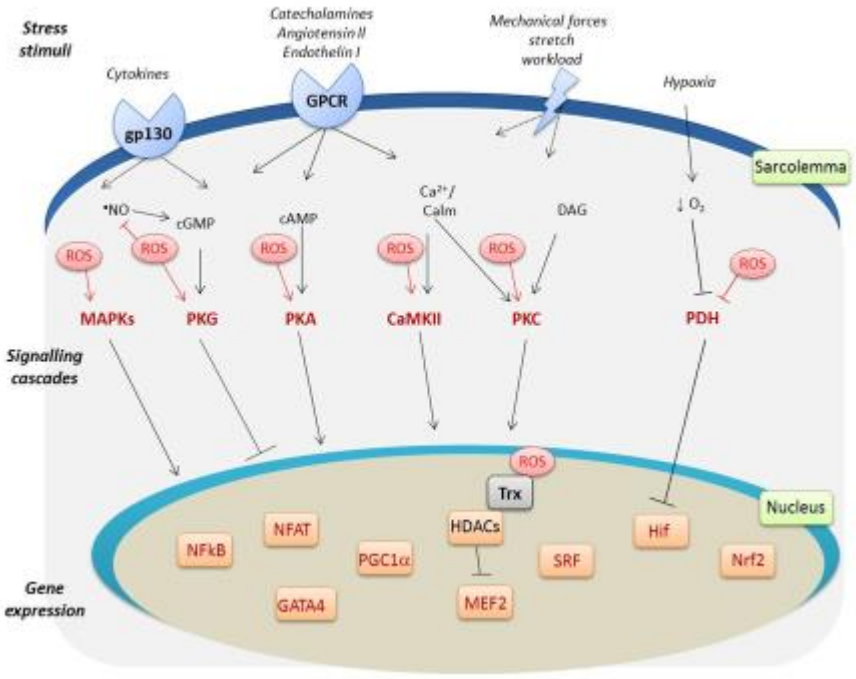

Fig. 2